EXHIBIT 22

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United States District Court,
E.D. Pennsylvania.

In re AVANDIA MARKETING, SALES PRACTICES AND PRODUCTS LIABILITY LITIGATION.
This Document Relates to All Actions.

Avandia MDL No. 1871.
|
No. 2007–MD–1871.
|
Jan. 4, 2011.

ORDER

CYNTHIA M. RUFE, District Judge.

***1 AND NOW,** on this 3rd day of January, 2011, upon consideration of GlaxoSmithKlines's Motions to Exclude the Testimony of Plaintiff Steering Committee Expert Witnesses Eliot A. Brinton, M.D. [Doc. No. 734], Nicholas P. Jewell, Ph.D. [Doc. No. 736] and Allan D. Sniderman, M.D. [Doc. No. 740], and for the reasons set forth in the attached memorandum opinion, it is hereby **ORDERED** that Defendant's Motions are **DENIED.**

It is so **ORDERED.**

MEMORANDUM OPINION AND ORDER

Presently before the Court are GlaxoSmithKline LLC's (GSK's) Motions to Exclude the Testimony of Plaintiff Steering Committee's Expert Witnesses Eliot A. Brinton, M.D.,[1] Nicholas P. Jewell, Ph .D.,[2] and Allan D. Sniderman, M.D.,[3] Plaintiffs' responses thereto, and GSK's replies. The Court has reviewed each expert's report and held a *Daubert* hearing to hear argument and testimony regarding the admissibility of the expert testimony on September 20–22, 2010. For the reasons set forth below, the Court will deny the motions to exclude the testimony of Drs. Brinton, Sniderman, and Jewell.

Factual Background

Plaintiff intends to offer Drs. Brinton, Jewell and Sniderman, among other experts, as generic expert witnesses for civil actions in MDL No. 1871. Their testimony will cover the alleged health risks involved in taking the drug Avandia, which is manufactured by GSK. GSK challenges the admissibility of this evidence, asserting that the experts used unreliable methods to reach their conclusions that Avandia may cause myocardial infarction in diabetic patients taking it to control their blood sugar.

Standard of Review

Federal Rule of Evidence 702 reads:

> [I]f scientific, technical or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient fact or data, (2) the testimony is the product of reliable principles and methods; and (3) the witness has applied the principles and methods reliably to the facts.

The Third Circuit has distilled this rule to two essential inquiries: 1) is the proffered expert qualified to express an expert opinion; and 2) is the expert opinion reliable?[4] In this case, GSK primarily challenges the reliability of the opinions.

Under the Third Circuit framework, the focus of the Court's inquiry must be on the experts' methods, not their conclusions. Therefore, the fact that Plaintiffs' experts and defendants' experts reach different conclusions does not factor into the Court's assessment of the reliability of their methods.[5] The experts must use good grounds to reach their conclusions, but not necessarily the best grounds or unflawed methods.[6]

Here, the scientific question the experts are addressing is whether there is a reasonable degree of scientific certainty that Avandia can cause myocardial infarctions. To meet

the *Daubert* standard, the experts must demonstrate that they have good grounds for their causation opinion (i.e. the opinion is based on methods and procedures of science, not subjective belief) and a reasonable degree of scientific certainty regarding their causation opinion. [7]

*2 Expert evidence must be relevant and reliable to be admissible. The Court must consider: 1) whether the expert's theory can be tested; 2) whether studies have been subject to peer review and publication; 3) the potential for error in a technique used; and 4) the degree to which a technique or theory (but not necessarily a conclusion) is generally accepted in the scientific community. [8] In cases such as this one, where the allegation is that a chemical (Avandia) causes a medical condition (myocardial infarction) experts should rely primarily on epidemiological studies to test their theory that the drug causes the disease. Double-blind randomized control trials, and particularly monotherapy trials comparing Avandia use to a placebo, are the "gold standard" of epidemiology. The best studies are designed and powered to test the outcome of interest (e.g., in this case, the most telling trial would be designed and have adequate subjects needed to study the impact of Avandia on the heart, not its effectiveness in managing blood sugar or other outcomes). [9]

Discussion

1. General Issues

Epidemiological Methods

The research on safety risks from Avandia use falls into three categories: 1) randomized control trials ("RCTs"), such as RECORD, DREAM, and ADOPT; 2) meta-analyses, such as NISSEN, SINGH, and MANUCCI; 3) and observational studies (such as the Harvard and Michigan studies). [10]

GSK argues that randomized control trials are the "gold standard" for epidemiological research, and that Plaintiffs' experts can find no support for their position in the RCTs conducted because the association between Avandia and myocardial infarction did not reach statistical significance in any of the RCTs. Therefore, GSK argues, the experts cannot rule out the possibility that the association was due to chance alone. In addition, GSK argues, none of the RCTs found Avandia to be associated with a statistically significant increase in atherosclerosis, which Plaintiffs' experts agree is the principal cause of myocardial infarction.

GSK also argues that Plaintiffs' experts do not give adequate weight to the findings of the RECORD study, which was a large RCT designed and carried out by GSK specifically to compare the cardiovascular safety of Avandia to that of Actos (a competitor medication in the same class). The RECORD study found no statistically significant increase in myocardial infarction, cardiovascular hospitalization or death.

Similarly, GSK argues that Plaintiffs improperly disregard the findings of the ADOPT and DREAM trials. Both are RCTs designed to test to the efficacy of Avandia for glycemic control, not its safety, and in both the association between Avandia and myocardial events approached but did not reach statistical significance.

Plaintiffs' experts each made specific criticisms about the RCT study designs and pointed out issues which complicate interpretation of the data, such as concurrent use of statins and a high drop out rate. These will be discussed in detail below. The experts also explained that when both the treatment group and the control group have a high background risk of myocardial infarction by reason of being diabetic, a large number of subjects is needed to adequately test whether Avandia is associated with an increased risk of myocardial infarction. If the sample size is too small to adequately assess whether the substance is associated with the outcome of interest, statisticians say that the study lacks the *power* necessary to test the hypothesis. Plaintiffs' experts argue, among other points, that the RCTs upon which GSK relies are all underpowered to study cardiac risks.

*3 To overcome the problem of underpowered studies, researchers may combine data from several studies into a meta-analysis. The NISSEN meta-analysis combined 42 clinical trials, including the RECORD trial and other RCTs, and found that Avandia increased the risk of myocardial infarction by 43%, a statistically significant result ($p = .031$). Plaintiffs point out that all the data used by Dr. Nissen in his meta-analysis came from GSK's own clinical trial registry. The NISSEN study was peer reviewed and published in the New England Journal of Medicine. Although GSK criticizes Plaintiffs' experts for relying on the NISSEN study, and notes that

meta-analysis generally can be unreliable, GSK points out no specific flaws or limitations in the design or implementation of the NISSEN meta-analysis, and the NISSEN results have been replicated by other researchers. For example, the SINGH meta-analysis pooled data from four long-term clinical trials, and also found a statistically significant increase in the risk of myocardial infarction for patients taking Avandia.[11] GSK and the FDA have also replicated the results of NISSEN through their own meta-analyses.

GSK argues that Plaintiffs' experts place too much reliance on meta-analysis (and particularly the NISSEN and SINGH studies), as meta-analysis is better for generating hypotheses than for testing them. While this may be true, the Court notes that if a statistically significant finding in a meta-analysis generates a hypothesis that Avandia is associated with a significant risk of heart attack, it may then become unethical to proceed with RCT of that substance, especially given the number of test subjects which would be required to adequately power a RCT to study whether Avandia causes heart attacks. Therefore, in some cases the science must proceed based upon less rigorous methods. This does not mean that inferences about causation cannot be made; it simply means that the expert must more carefully examine possible sources of bias or confounding and other factors which may make the study a weak indicator of causation.

Additionally, GSK argues that Plaintiffs' experts rely too heavily on observational studies, in which patients are not randomly assigned to treatment groups, and hence the patients for whom Avandia is prescribed may be different in some important ways from those in the control group who take another drug or no drug. One must carefully consider sources of bias, confounding, and alternative mechanisms.

Making Conclusions about Causation and the Bradford–Hill Criteria

Bradford–Hill criteria are used to assess whether an established association between two variables actually reflects a causal relationship.[12] Because these criteria are so well established in epidemiological research, it appears that the experts often consider these factors without citation to Bradford–Hill. When making causal inferences from associations between exposure to a chemical or drug and a disease outcome, the relevant Bradford–Hill criteria are: temporal relationship between the exposure and the outcome; the strength of the association between the exposure and the outcome; the dose-response relationship; replication of findings; the biological plausibility of or mechanism for such an association; alternative explanations for the association; the specificity of the association; and the consistency with other scientific knowledge. An expert need not consider or satisfy every criteria in order to support a causal inference. GSK argues that the Plaintiffs' experts equate association with causation and fail to apply the Bradford–Hill criteria when making causal inferences. The Court will examine this assertion in detail in the sections that follow.

**4 Although GSK asserts that a plausible biological mechanism to explain any association is one of the weaker Bradford–Hill criteria, GSK goes on to argue that Plaintiffs' experts lack a reliable theory for and proof of a biological mechanism of action. Specifically, they argue that the research on Avandia does not show that it causes a progression of atherosclerosis, the primary mechanism for myocardial infarction.[13]

As discussed in detail below, the Court finds that Plaintiffs' experts used reliable methods to find an association between Avandia and myocardial infarction, and adequately explored the Bradford–Hill criteria before drawing causal conclusions from that association

Study Selection

GSK argues that Plaintiffs' experts selectively reviewed studies which supported their causal inferences, and ignored studies which found no association between Avandia and adverse cardiac events. GSK argues that Plaintiffs' experts need to give detailed explanations regarding their decisions to rely on some studies and dismiss the importance of others. For example, the experts rely heavily upon the NISSEN and SINGH meta-analyses, but reject the MANNUCCI meta-analysis which found no correlation between Avandia usage and myocardial infarction. Of the twenty-three published observational studies, nine found a statistically significant increase in myocardial infarction for Avandia users, thirteen found no statistically significant correlation, and one showed a statistically significant protective effect. GSK argues that Plaintiffs' experts need to justify their reliance on the studies supporting their causal inference and rejection of the studies which are not supportive.

As the Court will discuss below, each of Plaintiff's experts adequately justified their reliance on some studies and rejection of others using scientific and statistical principles.

Statistical Significance

GSK criticizes Plaintiffs' experts for utilizing a clinical rather than a scientific standard of proof. Under a clinical standard, a doctor makes a risk-benefit analysis, whereas under a scientific standard one must have statistically significant findings to justify a causal inference. GSK also notes that Plaintiffs' experts rely on RCTs in which the positive correlation between Avandia and myocardial infarction does not reach statistical significance, such as DREAM, and ADOPT. Because the results are not statistically significant, the increased occurrence of myocardial infarction in the group taking Avandia may simply be due to chance. GSK argues that findings which are not statistically significant, although arguably *clinically useful*, are not *scientifically reliable*, and therefore do not meet the *Daubert* standard. In this case, while it is true that the experts point to the trend indicating increased risk found in many studies, only some of which reach statistical significance, the experts use the non-significant data only to bolster their inferences and not as their sole source of support. Therefore, the Court finds that the experts have sufficient statistically significant data to support their causal inferences, in combination with additional analysis.

The FDA

*5 Finally, GSK argues that the FDA has convened two advisory committee ("Ad Com") meetings regarding Avandia, in 2007 and 2010, and has opted not to vote on the question of whether Avandia *causes* myocardial infarction. Instead, the FDA has only stated that it has significant safety concerns about ischemic cardiac events for Avandia users. In 2007, the FDA Ad Com overwhelmingly voted "yes" when asked whether the available data supports the conclusion that Avandia increases cardiac ischemic risk in patients with Type II diabetes mellitus. After that Ad Com meeting, the FDA asked GSK to conduct a study comparing cardiovascular outcomes for Avandia versus Actos (the TIDE study). The FDA later suspended that study due to safety concerns about the risks associated with Avandia. Plaintiffs note that the Ad Com experts were not reassured by evidence presented to exonerate the drug in 2010, and the FDA took regulatory action to mitigate risk by significantly limiting the use of Avandia in the United States. The FDA's European counterpart suspended sales completely. Although the Court finds that the FDA did not vote on the precise question at issue here, that finding is not dispositive of the question as to whether Plaintiffs' experts meet the *Daubert* standard.

Defining Adverse Events

Finally, GSK argues that some of Plaintiffs' experts define "adverse event" too broadly, including myocardial ischemic events as well as myocardial infarctions. Plaintiffs' experts counter that myocardial ischemic events occur when there is a lack of oxygen to the heart muscle, and prolonged oxygen deprivation is the cause of myocardial infarction. Therefore, the difference between the two is a matter of degree; they are not caused by different mechanisms. In addition, Plaintiffs are forced, to some extent, to rely upon evidence regarding the broader category of myocardial ischemic events by the design of the studies (including those conducted by GSK) which broadly defined the outcomes of interest. However, by virtue of their expertise and the available data, the Court finds that the experts were able to draw reliable conclusions about myocardial infarction.

2. Expert Specific Issues

Eliot A. Brinton, M.D.

Dr. Brinton is a diabetologist and lipidologist, trained in endocrinology. He is primarily a clinical researcher and professor, employed by the University of Utah School of Medicine, but he also maintains a clinical practice. He once served on the Avandia Speakers' Bureau for GSK, but over time he became concerned with the lipid effects of Avandia, which were adverse compared to a similar drug (Actos), and eventually reached the conclusion that Avandia has the potential to cause cardiovascular disease. He continues to serve as a national advisor and speaker for GSK with regard to their lipid drug Lovaza, and has applied for grants from GSK to conduct research on Lovaza, but will no longer advocate for the use of Avandia based on his conclusions about the dangers of the drug.

*6 When Actos and Avandia were initially approved, Dr. Brinton stated that he had no preference for one over the other. Later he became aware that there were differences in lipid effects, and began to prescribe Actos significantly

more often in his practice.[14] The change in his use of Avandia was based not only on his clinical observations, but also on his review of the scientific research. Even before the NISSEN study was published, Dr. Brinton had reviewed the ADOPT and DREAM studies, which found cardiovascular detriment from Avandia use (albeit not a statistically significant detriment), and the PROactive study, which found cardiovascular benefits. After reading these studies, he had nearly stopped prescribing Avandia. Once the NISSEN study was published, he was puzzled by the FDA's decision to keep Avandia on the market, given that it had no meaningful advantage over Actos and was correlated with an increase in cardiovascular problems.[15]

Dr. Brinton's report focuses both on the adverse impact of Avandia on lipoprotein metabolism (a biological mechanism for ischemic heart disease, including myocardial infarction), and on the direct evidence that use of Avandia increases myocardial ischemic events.

Mechanism of Action

According to Dr. Brinton's report, one way that Avandia may cause myocardial infarction is by its effect on low-density lipoprotein (LDL) and apo B. While apo B levels are the strongest single predictor of atherosclerosis risk, 90% of apo B molecules are found contained in LDL particles, hence LDL (including LDL–P and LDL–C levels) is also often used to predict risk.[16] Another excellent predictor of risk is non-HDL cholesterol.[17] The standard predictor of cardiovascular disease risk is LDL–C.[18] Avandia studies report increases in LDL–C levels, generally in the 15–20% range on average, with some individuals showing even larger increases.[19] As statins are often prescribed for patients taking Avandia, the true effect of Avandia is probably underestimated in the research.[20]

Plasma apo B levels are increased in a dose-dependent manner by Avandia, by about 10% on average in studies not conducted by GSK.[21] LDL–P levels are also increased by Avandia usage. One published double-blind randomized control trial (DBRCT) found an 8% increase in LDL–P for patients taking Avandia (compared to a 4% *decrease* with Actos).[22] Non–HDL–C is increased by 20% or more with Avandia usage. Dr. Brinton rejects GSK's argument that Avandia's impact on LDL particle size is a mitigating factor, concluding that all three particles negatively effected are atherogenic, so even if Avandia does increase LDL particle size (a mitigating factor), the net effect of Avandia on lipids is negative.[23] He also evaluates GSK's arguments that the particle ratios are more important than the increase in negative particles, and that the approximately 5% increase of High–Density Lipoproteins (HDL) with Avandia usage is beneficial.[24] Dr. Brinton points out that there is no clearly established correlation between changes in the complex family of HDL molecules and a reduction in atherosclerosis and adverse events.[25] For example, Avandia decreases plasma apo A–1 levels, and it is those molecules, not the HDL itself, that appear to be responsible for the beneficial effect of HDL on atherosclerosis.[26] Avandia also reduces HDL–P.[27] Overall, Dr. Brinton notes, GSK simply does not have research findings to back its assertion that Avandia is linked to a favorable increase in HDL levels.[28] Furthermore, research on HDL-raising therapies reveals that some increases in HDL increase rather than decrease adverse cardiovascular events.[29]

*7 Similarly, Dr. Brinton talks about triglycerides (TC) as a mechanism by which Avandia increases the risk of cardiovascular disease, acknowledging that the mechanism for this relationship between triglycerides and atherosclerosis are not well understood.[30] Again, Dr. Brinton addresses GSK's arguments regarding this biological mechanism.[31]

Dr. Brinton discusses how Avandia usage increases levels of LpPLA2, which increase then destabilizes atherosclerotic plaques. The plaques are then vulnerable to rupture, causing myocardial infarctions.[32] LpPLA2 was discovered by GSK scientists, who are well aware of its role in coronary disease, but declined to study the impact of Avandia on LpPLA2 and to publish early studies which found Avandia increased LpPLA2.[33] Dr. Brinton evaluates GSK's position regarding LpPLA2, and gives detailed, research-based reasons for his disagreement.

Finally, Dr. Brinton discusses a well established connection between Avandia use and congestive heart failure (CHF).[34] The RECORD study revealed ten deaths from CHF in the Avandia group, and only two

in the control group. Dr. Brinton notes that CHF can contribute to myocardial ischemia. CHF impairs arterial blood flow, and can increase the likelihood and severity of ischemia to an area served by an atherosclerotic artery.[35] The combination of Avandia and insulin (a commonly prescribed, though off-label, combination) or Avandia and nitrates leads to an additive problem with CHF and is associated with increased myocardial ischemic events .[36]

Dr. Brinton also spends many pages in his report explaining why GSK's assertions of unchanged or reduced atherosclerosis with Avandia usage are somewhat misleading. For example, many patients taking Avandia are put on statins for associated increases in LDL–C.[37] Because statins are known to reduce atherosclerosis and cardiovascular disease events by about 30%, researchers may not see the true effects of Avandia usage on atherosclerosis.[38] In addition, some studies were underpowered to find effects on atherosclerosis (e.g., APPROACH).[39]

The Research Supporting an Inference of Causation

Dr. Brinton discusses which studies are best designed for reaching causal conclusions about Avandia's impact on the heart. He notes that the comparator treatment in a research study is an important consideration.[40] The impact of Avandia is clearest if the comparison group gets no treatment or a placebo. However, this scenario is not clinically relevant, as doctors rarely decide between prescribing Avandia and no treatment.[41] Therefore, direct comparison of Avandia to other glycemic control treatments is more clinically relevant, and most studies were designed to compare Avandia to other active drugs.

Dr. Brinton acknowledges that some clinical trials and observational data suggest that Avandia does not cause harm, but feels that the preponderance of data shows that it does increase cardiovascular disease events. He examines three types of evidence, beginning with RCTs and especially double-blind RCTs designed and powered to study cardiovascular effects. However, he notes again that even in a double-blind RCT, doctors may, and often do, prescribe statins to their patients in addition to Avandia or the control medication. The disproportionate use of statins in the Avandia arm of a trials can distort the rate of cardiac events in Avandia's favor.[42] Another disadvantage of using a DBRCT is that, because of the cost and complexity of conducting them, they are often inadequate in size to truly address the risk of serious but uncommon outcomes. Dr. Brinton also points out that many large RCT, such as ACCORD, ADVANCE, BARI–2D, and VADT, randomly assigned patients to a *treatment strategy* (intensive versus standard) but assignment to Avandia or another medication was not randomized. Therefore, to the extent that the researchers make findings as to the safety profile of Avandia from these studies, they should be considered observational studies and not the gold standard RCTs.

*8 Dr. Brinton discusses the RECORD trial (a RCT, but not a double-blind study) at length, and criticizes the lack of specificity in the endpoints of interest (for example, categorizing all deaths of unknown cause as cardiovascular deaths, for both the treatment and control arms of the study). He also notes that the prescribing doctors were permitted to measure patients' lipid profiles and even encouraged to prescribe statins. Statin use increased 9% more in the Avandia arm than the control arm. While this is clinically appropriate, it creates a serious problem in interpreting the study. Statin use (in both the treatment and control groups) also led to a lower-than-expected rate of cardiovascular disease overall in the study, which means that the study lacked statistical power. This problem with statistical power was compounded by the fact that a large percentage of study participants (in both arms) dropped out of the study.

Dr. Brinton gives a similarly detailed, scientific critique of the APPROACH, ADOPT, and DREAM studiesall studies with outcomes contrary to his opinion. He does not simply ignore these studies, as GSK suggests, but instead analyzes their strengths and weaknesses before concluding that they neither contradict nor undermine his opinion.

Next, Dr. Brinton turns his attention to meta-analyses of RCTs. A meta-analysis statistically combines studies, thereby increasing the statistical power so that researchers can study an infrequently occurring outcome of interest. Dr. Brinton points out two potential drawbacks of meta-analysis: 1) biased selection of studies; and 2) the results of one large trial can skew the overall findings. In 2007, the New England Journal of Medicine published the NISSEN meta-analysis, which combined results from 42 double-blind RCTs and found that patients taking Avandia had a statistically significant 43% increase in

myocardial ischemic events. NISSEN used *all* publicly available data from double-blind RCTs of Avandia in which cardiovascular disease events were recorded, thereby eliminating one major drawback of meta-analysis: the biased selection of studies. The SINGH, GSK and FDA meta-analyses replicated the key findings of the NISSEN study.[43] Meta-analyses combining studies which compared Avandia to a placebo, as opposed to an alternative treatment, showed a statistically significant 60% increase in myocardial ischemic events.

Dr. Brinton also points to the potential drawbacks of observational studies, including confounding and bias. These disadvantages, he notes, can be reduced by careful study design and execution. The advantage is that a much larger number of subjects can be studied using the observational method. Dr. Brinton reviewed twelve major observational studies, ten of which show statistically significant or nearly significant increases in major cardiovascular disease events for patients taking Avandia compared to the control groups. He cites to the strong evidence found in the Brownstein and Lipscombe studies, and discusses the limitations of the studies which did not find an association between Avandia and heart disease. One was cross-sectional, rather than longitudinal, in design and did not collect data which would allow researchers to control for socio-economic status, co-morbid conditions, existing health status, medical history, medication dose, and time on the drug.[44] The second, by Margolis, had a very wide confidence interval.[45]

***9** Dr. Brinton points out that the research (both meta-analytical and observational) shows that a significant increase in myocardial infarction and death occurs during the first six months of Avandia treatment when compared to other treatments.[46] He believes this supports his view that Avandia is an independent, causal factor.

GSK points out that Dr. Brinton did not take the position that Avandia causes heart attack until he was retained as an expert in this litigation. In fact, in 2007, Dr. Brinton recommended to the Utah State Medicaid Pharmaceutical and Therapeutics Committee that they keep both Actos and Avandia on their formulary, despite his observations about Avandia's negative impact on lipid profiles.[47] GSK argues that his current opinion is not reliable because it has changed since 2007. The Court finds that this criticism of Dr. Brinton goes to his credibility, and not to his methods. While a jury might find Dr. Brinton less credible because of his past position on Avandia, the opinions expressed in this case are based on reliable scientific methodology (the review of peer-reviewed, published studies and data using well established statistical and scientific principles).

GSK also argues that Dr. Brinton departed from scientific methodology by relying on data that is not statistically significant .[48] Although he did cite to studies in which the results were not statistically significant, his conclusions did not rest on those studies alone; rather, they were used to bolster the conclusions he drew from studies in which the findings were statistically significant. Similarly, the Court finds that Dr. Brinton found scientific evidence of an association, which he examined to rule out the effects of chance, bias and confounding, and then applied the Bradford–Hill criteria to reach a causal conclusion. GSK states that a biologically plausible mechanism is one of the weakest of the Bradford–Hill criteria, yet argues that the proven effects of Avandia on certain biomarkers does not necessarily translate into cardiovascular harm as Dr. Brinton hypothesizes. Because the Court finds that Dr. Brinton's hypotheses about plausible mechanisms are based on scientific data about both the links between Avandia and lipid profiles and the connections between lipid profiles and outcomes, and as one of several Bradford–Hill criteria (including consistency of findings, strength of association, dose response, temporal association), the Court does not find his analysis unreliable under *Daubert*.

Overall, the Court does not find that Dr. Brinton's conclusions were arrived at by "litigation-driven methodology" nor by his own clinical impressions, but rather by a thorough review and analysis of the published literature. When he rejects research that does not support his opinion, he explains why he finds that research flawed and not compelling. That is, his approach to the data was scientifically reliable. Any inconsistency in Dr. Brinton's opinions over time, and any flaws in his conclusions, go to weight, not admissibility.

Allan D. Sniderman, M.D.

***10** Dr. Sniderman is a cardiologist, medical researcher, and professor at McGill University. His research focuses on lipoprotein metabolism and, in particular, on the importance of apoB as a marker for vascular

disease. His work has been published in over 280 peer-reviewed publications. He believes that Avandia significantly increases myocardial ischemic events, including myocardial infarctions, and that the adverse changes it causes to apoB underlie a causal relationship. GSK does not challenge Dr. Sniderman's qualifications as a cardiologist, but does challenge his ability to analyze and draw conclusions from epidemiological research, since he is not an epidemiologist. GSK's briefs do not elaborate on this challenge, and in any event the Court finds it unconvincing given Dr. Sniderman's credentials as a researcher and published author, as well as clinician, and his ability to analyze the epidemiological research, as demonstrated in his report.

Dr. Sniderman begins by noting the undisputed claim that Avandia causes congestive heart failure through fluid retention. Once a heart begins to fail, even with therapy it can result in progressive deterioration.[49] When a medication like Avandia also increases LDL cholesterol and apo-B,[50] it may cause clinical events, including myocardial infarction.[51]

The reasoning behind Dr. Sniderman's causal conclusions rests upon his research on, and understanding of, the action of apoB lipoproteins. ApoB particles carry cholesterol and triglycerides from the liver and intestines to the rest of the body, and, according to Dr. Sniderman, provide a more accurate measure of the number of LDL particles in the system, and of cardiovascular risk, than measures of LDL cholesterol.[52] He cites to both epidemiological studies and research on the effect of statins to support his opinion that apoB is a better predictor of cardiovascular risk than LDL cholesterol.[53] He notes that in patients with Type 2 diabetes, LDL cholesterol is not generally elevated, but apoB is.[54] Therefore, in diabetics in particular, apoB is the best predictor of cardiac risk.

It is well documented by GSK's own research that Avandia use produces a statistically significant increase in LDL.[55] About one-third of patients studied experienced a substantial increase in LDL cholesterol, and another third a marked increase.[56] About one-third of patients studied experienced no increase in LDL cholesterol, but in some of those patients, apoB may increase even where overall LDL cholesterol levels are stable.[57] In these patients, apoB is a better indicator of increased risk.

According to Dr. Sniderman, researchers (including himself) have established that apoB particles gradually *cause* atherosclerosis (this process may occur over decades), and that atherosclerosis then can *cause* cardiovascular death. He asserts that this is not a theory about increased risk, but an established scientific fact.[58] GSK disputes this conclusion, but points only to dated sources for its position that authorities do not recognize apoB as a better predictor than LDL of cardiovascular disease.[59] GSK acknowledges that a causal role for LDL cholesterol in cardiovascular disease has been established and corroborated by controlled clinical trials.[60] And LDL is clearly raised in the majority of patients taking Avandia.

***11** GSK argues that increases in HDL with Avandia use mitigate any increase in LDL or apoB. Dr. Sniderman responds by noting that the mechanisms by which HDL decreases risk are not well understood, and medication-induced increases in HDL do not necessarily translate into clinical benefits.[61] He also notes that increases in HDL were not found in all Avandia studies, and some studies found no change or even a decrease in HDL with Avandia use.[62] In addition, he notes that previous research has not reported whether the same individuals who experience increased LDL also experience increased HDL with Avandia use, thus achieving or maintaining a health ratio.[63] Looking at patient level data obtained from GSK, Dr. Sniderman found changes in LDL and HDL cholesterol were frequently dissociated.

In reviewing the evidence that Avandia causes myocardial infarction, Dr. Sniderman is cognizant of the methodological limitations of various studies, including: careful and specific documentation of adverse outcomes, the use of low-risk subjects, and the use of statins in concert with Avandia and the control medications. Because these factors led to a small number of adverse events being recorded in either the treatment or the control arm of the study, most RCTs were underpowered to detect, at statistically significant levels, the relationship between Avandia and adverse cardiac outcomes.

It is for this reason that those with concerns about Avandia's impact on the heart (including GSK and the FDA) turned to meta-analysis, which combines RCTs to increase the power of the statistical analysis. Although there are problems inherent in using meta-analysis, independent researchers, GSK, and the FDA have all replicated the findings of the NISSEN study, which found a statistically significant increase in myocardial infarction for patients using Avandia. The consistency of the findings lends credence to the results. [64] Dr. Sniderman points to the SINGH study, which combined the very trials GSK relies upon to show that Avandia is safe (RECORD, DREAM, ADOPT, DARGIE) and found a statistically significant increase in the risk of myocardial infarction for Avandia users.

GSK criticizes Dr. Sniderman and other experts for selectively discussing meta-analysis which support their position, and ignoring studies like MANUCCI which do not find increased risk. But Dr. Sniderman describes and critiques the MANUCCI study in his report. [65] He notes that some of the studies were of very short duration: the meta-analysis included studies as short as 4 weeks in duration, which is a reasonable amount of time to study a medication's effectiveness, but not its risks. [66] It also is unknown how statin use was distributed between the experimental and control arms of the studies. [67] For these reasons and others, Dr. Sniderman rejects the results of the MANUCCI study.

Observational studies further confirm the finding that Avandia is associated with an increased risk of myocardial infarction and mortality. [68] Dr. Sniderman asserts that although these studies are subject to confounding and bias, the consistency of the findings across studies and the effect size is telling. [69]

***12** Finally, Dr. Sniderman turns to the RCTs. RECORD, DREAM, and ADOPT were designed and conducted by GSK. Although RCTs are generally considered the "gold standard" of research studies, they may still have methodological flaws. Dr. Sniderman opines that RECORD is *not* strong evidence that Avandia does not increase the risk of ischemic disease, because of: 1) a low event rate in both arms of the study; 2) the high dropout rate; 3) the failure to design and/ or power the study to assess the risk of myocardial ischemia; and 4) the confounding effect of concurrent statin treatment, which was not controlled by investigators (use of statins in the Avandia arm exceeded use in the control arm by 9%). [70] Even with the differential use of statins, the RECORD study showed a trend of increased cardiovascular events for those in the Avandia arm of the study. [71]

The DREAM and ADOPT studies were designed to study the impact of Avandia on pre-diabetics and newly diagnosed diabetics. Even in these relatively low-risk groups, there was a trend towards an adverse outcome for Avandia users (e.g., in DREAM, the p-value was .08, which means that there is a 92% likelihood that the difference between the two groups was not the result of mere chance). [72] It is not clear whether statin use was allowed in the DREAM study. The ADOPT study was marred by a very high dropout rate (more than 40% of the subjects did not complete the four year follow up) and the use of statins during the trial.

The Court finds that Dr. Sniderman examined studies which both supported and contradicted his conclusions in arriving at his opinions, he used findings which were not statistically significant only to bolster his opinion based on statistically significant findings, he properly considered the relationship between myocardial ischemic events and myocardial infarction, he evaluated the potential for bias and confounding, and he engaged in a Bradford–Hill analysis, with particular attention to biological mechanisms, strength and consistency of findings, and temporal issues. The Court further finds that Dr. Sniderman considered Avandia's effect on both apoB and LDL, as well as other aspects of cardiac health. Accordingly, the Court will deny GSK's Motion to Exclude his testimony, because the Court finds his opinion to be scientifically reliable.

Nicholas P. Jewell, Ph.D.

Dr. Jewell has a Ph.D. in mathematics from the University of Edinburgh, Scotland, and is an expert in biostatistics. He has been a professor of biostatistics at the University of California, Berkeley for the past 28 years. He authored a well-reviewed textbook entitled *Statistics for Epidemiology*, as well as over 100 peer reviewed papers on biostatistics. He has served as an expert in other cases, including cases regarding the adverse cardiovascular effects of Celebrex and Bextra.

GSK's Motion to Exclude Dr. Jewell as an expert witness is based on the following criticisms of his report: 1) failure to following scientific methodology in drawing causal inferences from associations; 2) failure to rule out the role of bias, confounding and chance; 3) failure to apply the Bradford–Hill criteria; 4) drawing conclusions about myocardial infarction from data measuring myocardial ischemic events; 5) failure to consider two recent meta-analyses which undermine his conclusions; 6) failure to consistently apply study evaluation criteria; and 7) failure to secure publication of his opinion; and 8) the lack of general acceptance by the relevant scientific community.

Failure to use proper methodology to draw causal inferences

***13** The Court's concern in deciding this *Daubert* motion is the methodology used, not the conclusions drawn, by the proposed experts. As noted, the experts are not required to use the best possible methods, but rather are required to use scientifically reliable methods. Plaintiffs and Defendants agree that to conclude that a medication causes an adverse outcome, the epidemiological data must show an association between the use of the medication and the adverse outcome, and that association must not be the result of chance, bias, or confounding. Once the researcher is confident that the association is real, he or she will assess other factors (such as the Bradford–Hill criteria) to draw conclusions about whether the medication which is associated with an outcome actually *causes* the outcome. GSK asserts that Dr. Jewell's methods were unreliable at all three steps. The Court disagrees.

Dr. Jewell's report includes a summary of several studies showing a statistically significant association between Avandia and myocardial infarction (i.e., an association that is unlikely to be found by chance). The Court disagrees with GSK's assertion that Dr. Jewell's opinion relies solely on the results of a single meta-analysis in which the association did not reach statistical significance, because his report clearly indicates a thorough review and consideration of a large number of studies. Dr. Jewell includes a thorough discussion of the methodological flaws in the design of and data collection for studies which do not find such an association, including an explanation about why those studies might be biased towards the null hypothesis (i.e. a statistical finding that the association may be the result of chance), including bias and confounding. While his report does not analyze the studies supporting his conclusions in the same detail, the Court notes that he places his greatest reliance upon those studies that minimize bias and confounding: 1) the RCTs, and particularly those in which the patients and doctors are blind to the study arm to which the patient is assigned, and those where the control arm is given a placebo rather than another active medication; [73] and 2) those studies that statistically or otherwise control for other variables. [74] Therefore, the Court finds that he has given attention to the role of chance, bias and confounding in arriving at his conclusion that there is a real association between Avandia and myocardial infarction, and further finds that he uses consistent criteria to evaluate the possible roles of chance, confounding, and bias both in studies that support and contradict his conclusions.

Although Dr. Jewell relies upon meta-analysis to reach his conclusion, he acknowledges that there are limitations to any meta-analysis. He explains that safety effects of a medication often cannot be determined without combining studies, because individual studies, especially drug efficacy studies as opposed to drug safety trials, are generally underpowered to explore unusual adverse effects, and may also be too short in duration. [75] , [76] He notes that he looked for more than one well-performed meta-analysis to lead to similar and consistent results before drawing his conclusions, to reduce the likelihood that the results were the result of chance, bias or confounding.

***14** GSK also argues that Dr. Jewell failed to consider the Bradford–Hill criteria in drawing his conclusions of causation from the association between Avandia and myocardial infarction. The Court again disagrees, finding that Dr. Jewell addressed many of the Bradford–Hill criteria throughout his report, including temporal relationships (evaluating studies as brief as four weeks and as long as several years), strength of association (which is seen in the confidence interval and the statistical probability of the association being the result of chance), replication of findings by other researchers, specificity of association (e.g., a showing that a similar association is not found for patients taking other drugs in its class), and consistency between studies using different methods (e.g., RCT and observational studies; studies using placebo controls and those using active controls). He notes that he could not assess whether there was a dose response, because there was little variation in the prescribed doses of Avandia. [77] Because he is a mathematician and not a

medical doctor, he did not examine biologically plausible mechanisms for the association.

Reliance on Studies with Over–Broad Outcome Measures

GSK objects to Dr. Jewell's reliance on studies with over-broad outcome measures in his report, while Dr. Jewell's report critiques GSK's safety studies, such as RECORD, on the same grounds, stating that GSK's use of an overly broad endpoint dilutes the signal strength for myocardial infarction and biases the results towards the null. [78]

GSK argues that much of the data upon which Dr. Jewell relies for his opinion regarding the causal relationship between Avandia and myocardial infarctions actually combines infarctions with other ischemic events. Some of these events are serious, but others are relatively minor. It is improper, then, to draw conclusions about infarctions from data about a broader category of events. The Court agrees with the need to focus, at this point in the litigation, on whether Avandia causes myocardial infarctions, but finds that Dr. Jewell's report does pinpoint the data on myocardial infarctions when it is possible to do so (e.g., in reviewing the NISSEN and SINGH meta-analyses, and the recent Harvard and Michigan studies), [79] and apparently finds in such data sufficient evidence to support his position. He does not try to extrapolate from the composite outcome data, as GSK argues, but rather looks to studies where myocardial infarctions are themselves measured outcomes. Plaintiffs also note that myocardial ischemic events and myocardial infarctions have the same underlying etiology (loss of oxygen to the heart), and an infarction is simply an ischemic event that deprives the heart of oxygen for a prolonged period of time. Accordingly, the Court does not find that the methods Dr. Jewell used to reach his conclusions were unreliable.

Ignoring Relevant Data

GSK argues that Dr. Jewell simply ignores relevant data which does not support his position, and in particular points to two meta-analyses conducted by GSK itself, neither of which has been peer reviewed or published. The Court notes that Dr. Jewell devotes much effort in his report to critiquing studies which do not support his position, including RECORD and the MANUCCI meta-analysis, [80] and also explains why he did not consider the two new meta-analyses performed by GSK to be persuasive. [81] He explains that in the first new meta-analysis, GSK researchers redefined the endpoint events, using an overly broad endpoint rather than focusing on myocardial infarctions, and did not engage in blind adjudication of outcome events. He also notes that the FDA found that study to be less reliable and informative than GSK's original meta-analysis. [82] In the second new meta-analysis, which expanded ICT from a meta-analysis of 42 studies to a meta-analysis of 52 studies, Dr. Jewell explains that a single study, APPROACH, dominated the data from the ten additional studies, as 95 of the 109 new events in the meta-analysis were in the APPROACH data set, and 5 of the 9 new myocardial infarctions occurred in that data set. [83] He found the APPROACH study to be unreliable for two primary reasons: 1) the study used an active medication for the control group, not a placebo; and 2) 76% of participants were on statins at the outset of the trial, whereas in the original studies, baseline statin use ranged from 3–11% in all but one of the 42 trials. [84] The Court is persuaded that Dr. Jewell did not simply ignore relevant data, but rather disregarded that data after finding it scientifically unreliable.

***15** Overall, the Court finds that Dr. Jewell's opinion is supported by his considered interpretation of the scientific data. The Court notes that his conclusions are not at issue at this time, but only his methods. The Court finds Dr. Jewell's methods are scientifically reliable, and accordingly will deny GSK's Motion.

Conclusion

Each of Plaintiffs' three experts have consulted an extensive body of epidemiological research to support their conclusions, and evaluated and weighed the quality and usefulness of the various studies. Although the conclusions differ from the conclusions reached by GSK's experts, generally speaking the epidemiological studies relied upon by Plaintiffs' experts are the same studies consulted by GSK and the FDA in their evaluation of the risk profile of Avandia. Plaintiffs' experts arrived at their conclusions that sound scientific evidence supports a causal inference without any speculative leap. They were able to opine to a causal connection between Avandia and myocardial infraction with a reasonable degree of medical or scientific certainty. Therefore, the Court finds that the experts' methods are the product of reliable principles and methods, and the experts had good grounds to reach their conclusions. Differences in conclusions go to the weight

of the evidence, and not to its admissibility. Accordingly, GSK's Motions to Exclude Plantiffs' general causation experts Drs. Brinton, Sniderman and Jewell are denied.

All Citations

Not Reported in F.Supp.2d, 2011 WL 13576

Footnotes

1 Doc. No. 734.

2 Doc. No. 736

3 Doc. No. 740

4 *In re TMI Litig.*, 193 F.3d 613, 664 (3d Cir.1999).

5 However, where the scientific community considers the evidence to be inconclusive, a difference of opinion may sometimes undermine the reliability of an expert's conclusion that there is a causal link, and may justify excluding that expert. *Magistrini v. One Hour Martinizing Dry Cleaning*, 180 F.Supp.2d 584, 607 (D.N.J.2002), *aff'd* 68 F. App'x 356 (3d Cir.2003).

6 *In re Paoli R.R. Yard PCB Litig.*, 35 F.3d 717, 745 (3d Cir.1994); *Holbrook v. Lykes Bros. S.S. Co.*, 80 F.3d 777, 784 (3d Cir.1996).

7 *See, Daubert v. Merrell Dow Pharms. Inc.*, 509 U.S. 579, 590 (1993).

8 *Daubert*, 509 U.S. at 593–94.

9 *In re Diet Drugs Prods. Liab. Litig.*, No. MDL 1203, 2001 WL 454586 at *13 (E.D.Pa. Feb. 1, 2001).

10 No RCT has found a statistically significant association between Avandia and myocardial infarction; the NISSAN and SINGH meta-analyses did find statistically significant associations, as did the majority of the observational studies. The MANNUCCI meta-analysis did not.

11 GSK complains that this study used interim RECORD data and unadjudicated ADOPT data.

12 *Soldo v. Sandoz Pharms. Corp.*, 244 F.Supp.2d 434, 514 (W .D. Pa.2003).

13 GSK cites to five long-term RCTs evaluating the impact of Avandia on atherosclerosis: VICTORY, STARR, APPROACH, PPAR, and HEDBLAD, none of which found an adverse impact, and MARGOLIS which found that Avandia *reduced* the risk of atherosclerotic disease of the heart by 40% (as statistically significant finding).

14 Brinton Report ("B.R.") at 7.

15 B.R. at 7.

16 B.R. at 9.

17 B.R. at 9.

18 B.R. at 9.

19 B.R. at 10.

20 B.R. at 10.

21 B.R. at 11.

22 B.R. at 11.

23 B.R. at 10–12.

24 B.R. at 14.

25 B.R. at 12–13.

26 B.R. at 14–15.

27 B.R. at 15.

28 B.R. at 16.

29 B.R. at 16.

30 B.R. at 16.

31 B.R. at 17.

32 B.R. at 18.

33 B.R. at 18.

34 B.R. at 20.

35 B.R. at 20–21.

36 B.R. at 21.
37 B.R. at 23.
38 B.R. at 23.
39 B.R. at 23–24.
40 B.R. at 4–5.
41 B.R. at 5.
42 B.R. at 26.
43 Dr. Brinton explains that a second meta-analysis performed by GSK, which included the APPROACH data, did not show a significant increase in cardiovascular disease with Avandia use, but because the APPROACH study recruited higher-risk patients, the data are not as strong as other data.
44 B.R. at 35–6 (discussing the Casscells study).
45 B.R. at 36.
46 B.R. at 19 (citing NISSEN and DORMUTH studies).
47 Doc. No. 734 at 1–3.
48 Doc. No. 734 at 3.
49 Sniderman Report ("S.R.") at 3.
50 Dr. Sniderman characterizes the evidence of this effect as "incontrovertible." S.R. at 3.
51 S.R. at 3.
52 S.R. at 11.
53 S.R. at 11.
54 S.R. at 13.
55 S.R. at 16–18.
56 S.R. at 17.
57 S.R. at 20.
58 S.R. at 7.
59 Doc. No. 740 at 16–19.
60 Doc. No. 740 at 17.
61 S.R. at 13–14.
62 S.R. at 21.
63 S.R. at 21.
64 S.R. at 26.
65 S.R. at 28–29.
66 S.R. at 28.
67 S.R. at 28.
68 S.R. at 30.
69 S.R. at 31.
70 S.R. at 31.
71 S.R. at 33.
72 S.R. at 33.
73 Jewell Report ("J.R.") at 10.
74 E.g., J.R. at 35.
75 J.R. at 5, 9.
76 Even studies such as RECORD, which are designed to test safety, may be underpowered if a large number of subjects drop out of their treatment group (as occurred in RECORD).
77 J.R. at 32.
78 J.R. at 8–9, 24.
79 J.R. at 11, 23, 36, 38.
80 J.R. at 24–32.
81 J.R. at 19–23.
82 J.R. at 20.

83 J.R. at 21.
84 J.R. at 22.

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EXHIBIT 23

Villous Atrophy and Negative Celiac Serology: A Diagnostic and Therapeutic Dilemma

Marisa DeGaetani, MD[1,2], Christina A. Tennyson, MD[1,2], Benjamin Lebwohl, MD, MS[1,2], Suzanne K. Lewis, MD[1,2], Hussein Abu Daya, MD[1], Carolina Arguelles-Grande, MD[1], Govind Bhagat, MBBS[3] and Peter H.R. Green, MD[1,2]

OBJECTIVES: **Patients with villous atrophy (VA) and negative celiac disease (CD) serologies pose a diagnostic and therapeutic dilemma. When a definitive etiology for VA is not determined, patients are characterized as having unclassified sprue (US), the optimal management of which is unknown.**

METHODS: **We studied adult patients with VA on biopsy and negative celiac serologies, evaluated at our tertiary referral center over a 10-year period. Testing for HLA DQ2/8 alleles, antienterocyte antibodies, giardia stool antigen, bacterial overgrowth, total serum immunoglobulins, and HIV was noted. Treatment, response, and repeat-biopsy findings were recorded.**

RESULTS: **The most common diagnoses of the 72 patients were seronegative CD, medication-related villous atrophy, and US. Of those with US, the majority reported symptomatic improvement with immunosuppressive therapy. Some patients initially labeled as unclassified were found to have VA associated with olmesartan use.**

CONCLUSIONS: **The role of medications in the development of VA and the optimal dose and length of immunosuppression for patients with US should be investigated further.**

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Introduction

Celiac disease (CD) is an immune-mediated disorder occurring in people genetically susceptible to gluten and results in varying degrees of villous atrophy (VA), crypt hypertrophy, and an increase in intraepithelial lymphocytes (1,2). However, these biopsy findings are not specific for CD. The diagnosis of CD is supported by positive antibody testing (tissue transglutaminase, deamidated gliadin peptide, and antiendomysial antibodies) as well as symptomatic and histologic response to a gluten-free diet (GFD). Most with CD have positive celiac serologies, but not all. Lesser degrees of VA are more frequently seen in seronegative CD patients (3). Genetic testing for the HLA alleles DQ2 and/or DQ8 supports the diagnosis (2).

Although CD is the most common cause of VA (4), patients with VA and negative celiac serologies are encountered, posing a diagnostic and therapeutic dilemma. Possible etiologies associated with VA and absent celiac serologies include common variable immunodeficiency (CVID), autoimmune enteropathy, small intestinal bacterial overgrowth, infection, intestinal lymphoma, collagenous sprue, Crohn's disease, and tropical sprue. VA can also result from certain medications. When celiac serologies are negative on a gluten-containing diet, alternative etiologies for VA should be considered before diagnosis of seronegative CD, to prevent an unnecessary lifelong GFD (5). At times, no definitive etiology for VA can be determined, and the patient is labeled as having unclassified sprue (US).

We analyzed our prospectively maintained Celiac Disease Center database to identify patients with seronegative VA. In addition to describing the various etiologies of seronegative VA, we also examined the response to treatment.

Methods

We report a series of adult patients with seronegative VA evaluated over a 10-year period (from 2001 to 2011) at a tertiary-care referral center. Patients included in this study were those with VA on duodenal

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biopsy and negative celiac serology (including tissue transglutaminase (TTG), deamidated gliadin peptide (DGP), and antiendomysial antibodies). Data regarding demographics, concomitant autoimmune disease, family history of CD, and initial biopsy results were collected. Testing was performed for human leukocyte antigen (HLA) DQ2/8 alleles, antienterocyte antibodies, giardia stool antigen, bacterial overgrowth, HIV, serum immunoglobulins, and T-cell receptor gene rearrangement. We sought the use of specific medications that have been involved with the development of VA, including mycophenolate mofetil and methotrexate; in light of the recently published case series from the Mayo Clinic describing an association between olmesartan and a sprue-like syndrome (6), we also identified patients taking this medication. Treatment, response, and repeat-biopsy results were recorded.

Patients in this study were considered to have seronegative CD if they had negative TTG, DGP, and antiendomysial antibody tests, positive genetic tests for CD, biopsy findings that were consistent with a diagnosis of CD (most notably intraepithelial lymphocytosis together with VA, which has been shown to be a sensitive marker for CD (7)), and response to a GFD (symptomatically and/or histologically), and tested negative for other etiologies of VA. As prior studies have demonstrated that the use of a single antibody test can underdiagnose CD (8,9), detection of more than one negative antibody test was looked for before a patient was labeled with seronegative CD.

Characteristic features used to diagnose patients with other etiologies of seronegative VA are as follows: CVID required decreased serum levels of at least two immunoglobulin subtypes in the setting of a normal albumin level, supported by biopsy findings consistent with CVID, including decreased plasma cells in the lamina propria. All patients with a diagnosis of giardiasis had positive giardia stool testing and responded symptomatically and/or histologically to treatment with metronidazole. Small intestinal bacterial overgrowth was diagnosed with positive hydrogen breath testing and symptomatic/histologic response to antibiotics. Medication-related VA was diagnosed after improvement in symptoms and/or histology once a medication reported to cause VA was discontinued. Diagnosis of collagenous sprue required a thickened subepithelial collagen band on histology. In the diagnosis of autoimmune enteropathy, we looked for a strong history of autoimmune disease and gut autoantibodies, supported by biopsy findings characteristic of this diagnosis, including increased plasma cells in the lamina propria, decreased goblet cells, and lack of increased intraepithelial lymphocytes. Patients with tropical sprue had traveled to an endemic area, had low B12 levels, and improved with antibiotics. A diagnosis of lymphoma required a monoclonal population of T cells. Patients were labeled with unclassified sprue (US) if they had no clear unifying diagnosis to account for their VA. Clinical response was defined as resolution of symptoms. Histologic response was defined as improvement in the degree of VA on duodenal biopsy.

This study was approved by our institutional review board.

Table 1. Demographics of patients with seronegative villous atrophy

Total *N*	72
Female (*N*, %)	37 (51.4%)
Mean age (years)	59 (range 29–85)
Presented with diarrhea (*N*, %)	55 (76.4%)
Mean time of follow-up (months)	26.4

Results

A total of 72 patients were identified with seronegative VA. All patients had been referred with a diagnosis of poorly responsive/refractory CD. Demographic information is provided in **Table 1**. Final diagnoses included: seronegative CD, 20 (28%); medication-related VA, 19 (26%); US, 10 (14%); CVID, 4 (6%); autoimmune enteropathy, 3 (4%); giardia, 3 (4%); $CD4^+$ small intestinal T-cell lymphoma, 3 (4%); tropical sprue, 3 (4%); bacterial overgrowth, 2 (3%); collagenous sprue, 2 (3%); enteropathy-associated T-cell lymphoma, 1 (1%); Crohn's disease, 1 (1%); and extensive gastric metaplasia, 1 (1%) (**Figure 1** and **Tables 2–5**).

The mean follow-up for all patients was 26.4 months. Two patients died of unrelated causes, two died of complications of their disease (intestinal failure related to extensive gastric metaplasia and transformation of $CD4^+$ small intestinal T-cell lymphoma to an aggressive form), and two developed lymphoproliferative disorders (multiple myeloma and B-cell lymphoma).

Seronegative CD was the most common diagnosis in our study, seen in 20 of 72 patients (28%). This was followed by medication-related VA, largely as a result of olmesartan use (16 of 19 patients in this category). The other culprit medications were mycophenolate mofetil (2 patients)

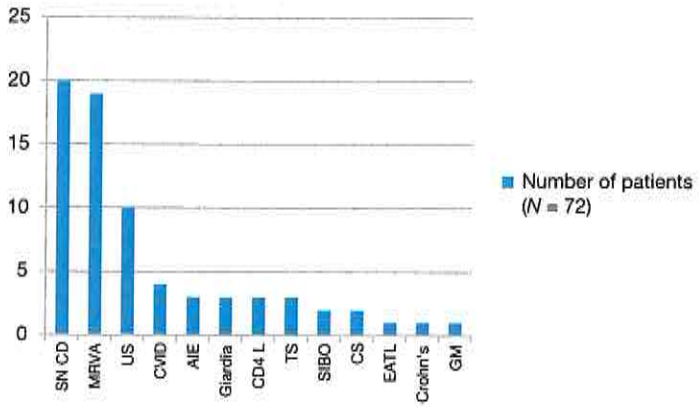

Figure 1. Etiologies of seronegative villous atrophy. AIE, autoimmune enteropathy; CD4 L, $CD4^+$ T-cell lymphoma; CS, collagenous sprue; CVID, common variable immunodeficiency; EATL, enteropathy-associated T-cell lymphoma; GM, gastric metaplasia; MRVA, medication-related villous atrophy; SIBO, small intestinal bacterial overgrowth; SN CD, seronegative celiac disease; TS, tropical sprue; US, unclassified sprue.

Table 2. Seronegative celiac disease

No.	Age (y)/sex	Fam Hx CD	AI dz	SIBO?	Culprit meds	Ig defic.	Giardia	A-E Ab	HLA DQ2/8	TCR	Degree VA initial bx	Clinical improv./ GFD	Degree of VA f/u biopsy	F/u bx improv.?	Required immuno-suppression	F/u time after initial visit (mo.)
1	59/M	–	–	NT	–	–	–	–	+	NT	PVA	+	NA	NA	–	12
2	36/F	–	–	NT	–	–	–	NT	NT	+ Clonal	DNS	+	Normal	+	–	22
3	55/F	+	–	NT	–	–	NT	NT	+	NT	PVA	+	NA	NA	–	72
4	44/M	+	+	NT	–	–	NT	NT	+	NT	PVA	+	Normal	+	–	36
5	48/F	–	+	+	–	–	–	NT	NT	–	TVA	+	PVA	+	–	60
6	43/F	–	–	NT	–	–	–	NT	+	NT	PVA	+	Normal	+	–	36
7	33/M	+	–	NT	–	–	NT	NT	+	NT	STVA	+	NA	NA	–	96
8	42/M	–	–	NT	–	IgM	NT	NT	+	–	PVA	+	Normal	+	–	12
9	72/M	–	–	NT	–	–	NT	–	+	NT	PVA	+	NA	NA	–	4
10	29/M	–	–	NT	–	–	NT	–	NT	NT	STVA	+	NA	NA	–	5
11	72/M	–	–	NT	–	–	–	Weak +	+	NT	TVA	+	NA	NA	–	10
12	40/F	–	–	+[a]	–	–	NT	NT	+	NT	PVA	+	NA	NA	–	24
13	64/M	–	–	NT	–	–	NT	NT	+	NT	PVA	+	NA	NA	–	12
14	67/F	–	+	+[a]	–	IgM	–	NT	+	NT	TVA	+	Normal	+	–	12
15	58/M	–	–	NT	–	–	–	NT	NT	NT	PVA	+	NA	NA	–	3
16	70/M	–	–	NT	–	–	NT	–	+	–	DNS	+	NA	NA	+	6
17	60/F	+	–	NT	–	–	NT	–	+	–	PVA	+	NA	NA	+	108
18	70/M	–	–	–	–	IgM	NT	NT	NT	NT	STVA	+	Normal	+	+	24
19	62/F	–	–	+	–	IgM	NT	NT	+	–	STVA	+	PVA	+	+	36
20	47/F	–	–	–	–	IgM	–	–	+	–	DNS	+	NA	NA	+	5

A-E Ab, anti-enterocyte antibody; AI dz, autoimmune disease; bx, biopsy; CD, celiac disease; defic., deficiency; DNS, degree of villous atrophy was not specified; Fam Hx, family history; f/u, follow-up; GFD, gluten-free diet; HLA DQ2/8, human leukocyte antigen DQ2/8 alleles; improv., improvement; NA, not applicable; No., patient number; NT, not tested; PVA, partial villous atrophy; SIBO, small intestinal bacterial overgrowth; STVA, subtotal villous atrophy; TCR, T-cell receptor; TVA, total villous atrophy; VA, villous atrophy.
[a]Remote history of treated small intestinal bacterial overgrowth.

Table 3. Medication-related villous atrophy

No.	Age (y)/sex	HLA DQ2/8	Culprit meds?	Degree VA initial bx	Increase subepithelial collagen	Increase IEL on bx	GFD	Clinical improv./ GFD	Abx	Clinical improv./ Abx	IS	Clinical improv./ IS	Relapse off IS?	Clinical improv. after stopping med	Bx improv. after stopping med	F/u time (mo.)
1	61/M	+	Olmesartan	TVA	+	+	+	–	+	?	+	+	+	+	NA	24
2	73/F	+	Olmesartan	TVA	+	+	+	–	–	NA	+	+	+	+	NA	21
3	82/M	NT	Olmesartan	PVA	+	+	+	–	+	?	+	+	+	+	NA	66
4	63/M	+	Olmesartan	STVA	+	+	+	–	–	NA	+	+	+	+	NA	50
5	69/F	–	Olmesartan	TVA	+	+	+	–	–	NA	+	+	+	+	+	12
6	66/M	+	Olmesartan	TVA	+	+	+	–	+	+	+	+	+	+	+	12
7	75/F	+	Olmesartan	DNS	+	+	+	–	+	+	+	+	+	?	NA	6
8	63/F	+	Olmesartan	TVA	+	+	+	–	–	NA	+	+	+	+	NA	42
9	52/M	NT	Olmesartan	STVA	–	–	+	–	+	–	+	+	+	+	NA	18
10	58/F	+	Olmesartan	PVA	+	–	+	–	–	NA	+	+	+	+	NA	24
11	83/M	+	Olmesartan	DNS	–	+	+	–	+	–	+	+	+	+	NA	11
12	67/F	+	Olmesartan	PVA	+	–	+	–	+	–	+	+	+	+	NA	8
13	75/M	+	Olmesartan	TVA	–	–	+	+	–	NA	+	+	+	+	NA	21
14	68/F	+	Olmesartan	TVA	–	+	+	–	+	–	+	+	+	+	NA	36
15	62/M	+	Olmesartan	TVA	+	+	+	–	+	–	+	+	+	+	NA	10
16	64/F	NT	Olmesartan	DNS	–	–	+	–	–	NA	+	+	+	+	NA	12
17	74/F	+	MMF	PVA	+	–	+	–	–	NA	NA	NA[a]	NA	+	NA	7
18	57/F	NT	MMF	PVA	–	–	–	NA	–	NA	NA	NA[a]	NA	+	NA	3
19	67/F	NT	Methotrexate	PVA	–	–	+	+	–	NA	NA	NA[a]	NA	+	+	120

Abx, antibiotics; bx, biopsy; f/u, follow-up; GFD, gluten-free diet; HLA DQ2/8, human leukocyte antigen DQ2/8 alleles; IEL, intraepithelial lymphocytes; improv., improvement; IS, immunosuppression; MMF, mycophenolate mofetil; NA, not applicable; No., patient number; NT, not tested; PVA, partial villous atrophy; STVA, subtotal villous atrophy; TVA, total villous atrophy; VA, villous atrophy.
[a]Culprit meds were immunosuppressive agents.

and methotrexate (1 patient). Twelve of the patients with medication-related VA had biopsies consistent with collagenous sprue (11 olmesartan and 1 mycophenolate). US was seen in 10 of 72 patients (14%); 70% were female, and the mean age was 62 years (range 33–82). All US patients were symptomatic at presentation, diarrhea being the main complaint in 70%. Three patients had a history of autoimmune diseases, none had a family member with CD, and 70% were positive for HLA DQ2/8. Initial biopsy revealed partial villous atrophy in 4 of 10 patients, subtotal villous atrophy in 1 of 10, and total villous atrophy in 4 of 10. The degree of VA was not specified in 1 patient. Increased intraepithelial lymphocytes were seen in 90% of patients. The results of additional diagnostic studies are shown in **Table 5**. One patient (patient 8) subsequently developed a low-grade B-cell lymphoma in the bone marrow with no evidence of small intestinal involvement.

Eight patients with US were tried on a GFD, but only 2 patients (25%) experienced initial symptomatic improvement. One patient showed no histologic improvement on repeat biopsy, and the other showed some degree of histologic improvement; however, repeat biopsy was performed only after immunosuppressive therapy was started. Response to a GFD is not a good predictive factor for CD, as it has not been shown to be a specific test for CD (10,11). Six patients were treated with antibiotics, and one reported improvement in symptoms (patient 9). She was treated with rifaximin for 10 days and experienced temporary improvement of her diarrhea. Notably, the VA on her repeat biopsy had improved before antibiotic therapy, but histology remained abnormal. Eighty percent received immunosuppressive agents, and 86% of these showed symptomatic improvement in follow-up data. Medications included budesonide, beclomethasone, prednisone, azathioprine, and 6-mercaptopurine. Of these patients, all experienced improved symptoms within 2 months. Only one patient (patient 5) was able to successfully stop treatment after 4 months of therapy. One patient (patient 1) had no response to any treatment, and one patient was lost to follow-up after immunosuppressive therapy was started.

Discussion

Seronegative VA is uncommon; however, it is important to be able to differentiate between seronegative CD and other causes of VA, as their prognosis and treatment are distinct, despite their often having similar clinical presentations and biopsy findings. Of the 72 patients seen over a 10-year period at our single tertiary-care referral center, we found a definitive etiology for seronegative VA in approximately 85%. An interesting finding in our series was the number of patients who were initially labeled with unclassified sprue who were ultimately found to have VA as a result of olmesartan use.

The most common diagnosis seen in our series was seronegative CD. Antibody testing using TTG IgA and endomysial IgA is highly sensitive and specific for CD, whereas antigliadin antibody testing is not (13–15). The newer DGP assay is also highly sensitive and specific for CD and has been shown to detect patients who were seronegative by TTG testing (16); however, only one of the patients with seronegative CD in our study was tested for antibodies to DGP. Even in IgA deficiency, most patients can still mount an IgG antibody response to TTG and gliadin (17,18). However, in the presence of partial VA or silent or subclinical CD, antibody testing may be negative (3,19). Our seronegative CD patients all had histologic findings and responded symptomatically to treatment with a GFD, and all eight who underwent repeat biopsy at the time of clinical improvement had improved histology.

The second largest group comprised those with VA from medication use. Specific medications, including methotrexate, mycophenolate mofetil, and azathioprine, have been considered to cause VA (20–24), as well as the angiotensin receptor blocker olmesartan, as reported in 22 patients from the Mayo Clinic (6). These authors also reported an association between olmesartan and collagenous sprue (25). We identified 16 patients taking olmesartan, of whom 68% had increased subepithelial collagen in addition to VA. Upon discontinuation of this medication, all 15 patients on whom we had follow-up data improved symptomatically, no longer requiring immunosuppressive therapy if they had previously been on it (budesonide, prednisone, and azathioprine), and some have resumed a gluten-containing diet with no recurrence of symptoms. Notably, one patient who had symptomatic improvement off olmesartan was then rechallenged with the medication and symptoms recurred. The role of olmesartan and other angiotensin receptor blockers in enteropathy needs to be investigated further.

Unclassified sprue (US), a diagnosis of exclusion, was seen in 14% of our patients. Although this result is lower than that reported by Pallav *et al.*, who diagnosed unspecified enteropathy in 10 of 30 of their patients with seronegative VA (10), before identifying olmesartan as a cause of VA, we too had considered 30% of our seronegative patients to have US. Patients with US all lacked an alternate single unifying diagnosis; however, HLA DQ2/8 alleles were found in 70% of those tested. Most patients responded to treatment with immunosuppressive therapy. In our study, only one patient had an isolated deficiency of serum IgG as in the series of Pallav *et al.* (10). Yet two US patients had isolated low levels of IgM, and 12 patients with a known etiology for their VA had an isolated IgM deficiency. The significance of detecting low IgM levels in patients presenting with sprue-like disease is unclear (26), though an association between IgM deficiency and CD has been reported (27).

Although eight patients were found to have small intestinal bacterial overgrowth by breath testing at the time of evaluation for their VA, in only two was it considered to be the sole cause of their VA. One patient with total VA was found to have enteropathy-associated T-cell lymphoma, a large-cell high-grade non-Hodgkin's lymphoma (28), responsible for the VA without evidence of CD. Additionally, we identified three patients who were referred for poorly responsive CD and found to have VA due to an intestinal lymphoma that primarily involved the lamina propria, which was distended by an infiltrate of small-sized $CD4^+$ T cells. This is a rare type of primary intestinal T-cell lymphoma, with only a few reported cases describing prolonged survival, which can histologically mimic but is not associated with CD (29).

Table 4. Other causes of villous atrophy

Diagnosis	Age (y)/ sex	Fam Hx of CD	AI dz	SIBO?	Culprit meds?	Ig defic.	Giardia	A-E Ab	HLA DQ2/8	TCR	Degree VA initial bx	GFD	Clinical improv/ GFD	Abx	Clinical improv/ Abx	IS	Clinical improv/ IS	Degree VA f/u bx	F/u bx improv?	F/u time (mo.)
CVID	45/F	–	–	+	–	IgA, IgG, IgM	–	NT	+	–	DNS	+	–	+	+	+	?	PVA	–	3
CVID[a]	60/M	–	–	NT	–	IgA, IgG, IgM	+	NT	+	–	DNS	+	–	+	+	–	NA	Normal	+	12
CVID	73/M	–	–	NT	–	IgG, IgM	–	–	+	–	PVA	+	–	–	NA	+	+	PVA	–	30
CVID	29/F	+	+	NT	–	IgA, IgG, IgM	–	–	+	NT	PVA	–	NA	–	NA	+	+	NA	NA	6
AIE	66/F	–	–	NT	–	–	NT	+	–	NT	PVA	–	NA	–	NA	–	NA	NA	NA	5
AIE	59/M	–	–	NT	–	–	–	+	+	–	TVA	+	–	–	NA	+	+	TVA	–	12
AIE	59/M	+	–	NT	–	IgM	–[b]	–	+	NT	PVA	–	NA	+	+	+	+	NA	NA	6
Giardia	54/M	–	–	–	–	–	+	–	+	–	TVA	+	–	+	+	–	NA	Normal	+	12
Giardia	41/M	–	–	NT	–	–	+	NT	–	NT	TVA	+	–	+	+	–	NA	STVA	+	2
Giardia	32/M	–	–	–	–	–	+	NT	NT	NT	PVA	+	–	+	+	–	NA	Normal	+	60
CD4 lymphoma	51/F	+	–	–	–	–	NT	–	–	+ Clonal	STVA	+	–	–	NA	+	+	STVA	–	3
CD4 lymphoma	46/M	–	–	–	–	–	–	NT	+	+ Clonal	TVA	+	–	+	–	+	+	TVA	–	144
CD4 lymphoma	70/M	–	–	–	–	–	–	NT	+	+ Clonal	STVA	+	–	–	NA	+	+	STVA	–	30
SIBO	50/F	–	–	+	–	–	–	NT	+	NT	PVA	+	–	+	+	–	NA	Normal	+	1
SIBO	70/F	–	–	+	–	–	–	NT	–	–	TVA	+	–	+	+	+	+	Normal	+	84
Tropical sprue	72/M	–	–	NT	–	IgM	–	NT	+	–	PVA	–	NA	+	+	–	NA	PVA	–	16
Tropical sprue	40/F	–	–	NT	–	–	–	NT	+	NT	PVA	+	–	+	+	–	NA	NA	NA	6
Tropical sprue	85/F	–	–	NT	–	–	–	–	+	–	STVA	+	–	+	+	+	–	STVA[c]	–	12
Collagenous sprue	34/F	+	–	NT	–	–	NT	NT	NT	–	TVA	+	+	+	?	+	+	PVA	+	36
Collagenous sprue	63/M	+	+	NT	–	–	NT	–	NT	+ Clonal	TVA	+	+	–	NA	+	+	TVA	–	12
T-cell lymphoma	50/M	–	–	NT	–	–	NT	NT	+	+ Clonal	DNS	+	–	+	+	–	NA	NA	NA	12
Gastric metaplasia	68/F	–	+	NT	Methotrexate	IgM	–	NT	+	NT	TVA	+	–	+	–	+	–	TVA	–	12
Crohn's disease	76/M	–	+	NT	–	–	–	–	+	–	PVA	+	–	–	NA	+	?	Normal	+	72

Abx, antibiotics; A-E Ab, anti-enterocyte antibody; AI dz, autoimmune disease; AIE, autoimmune enteropathy; bx, biopsy; CD, celiac disease; CVID, common variable immunodeficiency; defic., deficiency; DNS, degree of villous atrophy was not specified; Fam Hx, family history; f/u, follow-up; GFD, gluten-free diet; HLA DQ2/8, human leukocyte antigen DQ2/8 alleles; improv., improvement; IS, immunosuppression; NA, not applicable; NT, not tested; PVA, partial villous atrophy; SIBO, small intestinal bacterial overgrowth; STVA, subtotal villous atrophy; TCR, T-cell receptor; TVA, total villous atrophy; VA, villous atrophy.

[a]Although the patient had positive giardia testing and improved with antibiotics, the initial biopsy had findings consistent with autoimmune enteropathy, and so he was labeled as such, although it is likely that two processes were contributing to his presentation.

[b]Patient had biopsy consistent with autoimmune enteropathy.

[c]Biopsy done after immunosuppressive therapy but before antibiotic therapy.

To our knowledge, our series of seronegative VA is the largest study to date. However, given that our population was composed of referrals made to a tertiary-care center, it may not be an accurate representation of seronegative VA in general, which may contain a higher proportion of seronegative CD that is responsive to a GFD. Additionally, as the patients were seen by four different physicians over a 10-year period, different patterns of testing for the work-up of

Table 5. Unclassified sprue

No.	Age (y)/sex	Fam Hx of CD	AI dz	SIBO	Culprit meds	Ig defi-ciency	Giar-dia	A-E Ab	HLA DQ2/8	TCR	Degree VA initial bx	IEL on bx	GFD	Clinical improv/ GFD	Abx	Clinical improv/ Abx	IS	Clinical improv/ IS	Degree VA f/u bx	F/u bx im-prov.?	F/u time (mo.)
1	82/F	–	–	+	–	–	–	–	–	–	DNS	+	+	–	+	–	+	–	PVA	–	6
2	65/M	–	–	NT	–	IgM	NT	NT	+	NT	TVA	+	–	NA	+	–	+	+	NA	NA	1
3	67/F	–	–	+	–	–	–	Weak +	+	–	TVA	+	+	–	+	–	+	+	TVA	–	4
4	40/M	–	–	+	–	–	–	–	+	–	STVA	+	+	–	+	–	+	+	NA	NA	1
5	79/F	–	–	NT	–	IgG	–	NT	+	–	TVA	+	+	+	–	NA	+	+	STVA	+	6
6	78/M	–	+	NT	–	–	NT	–	+	–	TVA	–	+	–	–	NA	+	+	N/A	NA	1
7	62/F	–	–	–	–	IgA	NT	–	–	–	PVA	+	–	NA	+	–	+	?	PVA	–	2
8	65/F	–	+	–	–	IgA	–	–	+	–	PVA	+	+	–	–	NA	+	+	PVA	–	36
9	33/F	–	–	NT	–	–	–	NT	+	+ Clonal	PVA	+	+	–	+	+	–	NA	Nor-mal[a]	+	24
10	49/F	–	+	–	–	IgM	–	NT	–	NT	PVA	+	+	+	–	NA	–	NA	PVA	–	132

Abx, antibiotics; A-E Ab, anti-enterocyte antibody; AI dz, autoimmune disease; bx, biopsy; CD, celiac disease; DNS, degree of villous atrophy was not specified; Fam Hx, family history; f/u, follow-up; GFD, gluten-free diet; HLA DQ2/8, human leukocyte antigen DQ2/8 alleles; IEL, intraepithelial lymphocytes; improv., improvement; IS, immunosuppression; NA, not applicable; No., patient number; NT, not tested; PVA, partial villous atrophy; SIBO, small intestinal bacterial overgrowth; STVA, subtotal villous atrophy; TCR, T-cell receptor; TVA, total villous atrophy; VA, villous atrophy.
[a]Biopsy before antibiotic therapy started.

seronegative VA were noted. One area of testing that was poor was testing for HIV. Ideally, in all cases clinical response needs to be confirmed by histologic improvement on scheduled follow-up biopsies; however, we could not document this in all cases, as some patients were lost to follow-up or did not wish to undergo repeat biopsy.

Proposed work-up for seronegative VA. On the basis of our results, we propose that all patients with seronegative VA should initially be tested for HLA DQ2 and DQ8. If the test is negative, we would usually exclude CD. Immunoglobulin deficiency should also be excluded, both selective IgA deficiency and CVID. A thorough history should be obtained, which should include medication and travel history. We recommend further testing for antienterocyte antibodies, giardia antigen in the stool, and HIV, and breath testing for small intestinal bacterial overgrowth. If there is suspicion of lymphoma based on the pathological appearance, further studies including immunohistochemistry and testing for T-cell receptor gene rearrangement to identify occult lymphomas can be performed. Previous and initial biopsies should be obtained and reviewed by an experienced gastrointestinal pathologist in order to elucidate a potential etiology based on certain biopsy characteristics, for example, occult lymphoma and eosinophilic enteritis. If no clear etiology can be determined, and a patient is believed to have US, we recommend treatment based on the severity of the patient's illness—initially corticosteroids, such as budesonide and/or prednisone, with other immunosuppressive agents if necessary. The best agent, as well as the optimal treatment time, is unknown and needs to be researched further; however, our experience with budesonide would favor it as a first-line therapy (30). Patients with medication-related VA typically require immunosuppressive treatment for a period of time.

VA with negative celiac serology is uncommon. While most patients with seronegative VA in our study had either CD or other identifiable disease processes, some patients had VA of unclear etiology (US) that did not respond to a GFD alone and required immunosuppression.

CONFLICT OF INTEREST

Guarantor of the article: Peter H.R. Green, MD.

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